8EHQ-0801-14984

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CERTIFIED MAIL

July 31, 2001

TSCA Document Control Office (7408)
Office of Pollution Prevention and Toxics
Environmental Protection Agency
1200 Pennsylvania Avenue NW
Washington DC 20460

Attention: TSCA 8(e) Coordinator

RE: **tert-Butyl Hydroperoxide (TBHP; CASRN 75-91-2) - Skin Sensitization Study with TBHP-70 (70% TBHP in solution) in Guinea Pigs (Maximization Test)**

Dear Sir or Madam:

Lyondell Chemical Company (Lyondell) has received preliminary results from a study examining possible sensitizing properties of tert-Butyl hydroperoxide (TBHP; CASRN 75-91-2) in the guinea pig. A maximization test was conducted according to EC Directive 96/54/EC (Method B.6) and OECD Guideline No. 406. Lyondell is submitting this information in accordance to Section 8(e) of the Toxic Substances Control Act (TSCA) and EPA's 1991 Section 8(e) Reporting Guide because it includes a finding that EPA may consider to be 8(e) reportable. Lyondell has not made a determination as to whether a significant risk of injury to health or the environment is actually presented by the findings.

The information received was in the form of a draft report. The study is being conducted at TNO Nutrition and Food Research in the Netherlands and is sponsored by Lyondell Chemical Europe for the EU risk assessment of TBHP. As part of the study, 10 male guinea pigs were administered an induction treatment by intradermal injections of a 1% test dilution of TBHP-70 in physiological saline followed one week later by a topical application of a 30% test dilution of TBHP-70 in physiological saline. The challenge treatment, administered 14 days after the last induction, consisted of a topical application of a 3% test dilution of TBHP-70 in physiological saline. The challenge treatment with TBHP-70 showed positive signs of sensitization in six of ten guinea pigs. Based on the preliminary results, it has been concluded that TBHP-70 is a sensitizer. For your review, a copy of the draft report is attached. We will forward the final report when it is completed.

Specific questions concerning this submission should be directed to my attention at 713-309-2136. Thank you for your assistance in this regulatory matter.

Sincerely,

Patrick L. Gibson
Product Safety Specialist - Regulatory
Corporate TSCA Coordinator
Lyondell Chemical Company

8EHQ-01-14984

Contains NO CBI

Enclosure

88010000195

(1)

*TNO report*
V3413/07

# Sensitization study with TBHP-70 in guinea pigs (maximization test)

TNO Nutrition and Food Research

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Date:
July 2001

Author(s):
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At the request of:
Lyondell Chemical Europe, Inc., Lyondell Chemical House
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Great Britain

TNO Project number:
42113/01.07

TNO Study code:
3413/07



Study director:
M.K. Prinsen

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Draft

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None

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2

## Summary

1. The test material **TBHP-70** was examined for possible sensitizing properties by a maximization test in guinea pigs, according to EC Directive 96/54/EC, method B.6 and OECD Guideline no. 406, using 10 test animals and 5 controls.

2. The test comprised:
   Test animals
   - induction treatment by intradermal injections of Freund's Complete Adjuvant (FCA) 1:1 diluted with isotonic saline, a 1% test dilution of **TBHP-70** in physiological saline, and a 1% test dilution of **TBHP-70** in FCA, followed one week later by topical application of a 30% test dilution of **TBHP-70** in physiological saline,
   - challenge treatment, 14 days after the last induction, by topical application of a 3% test dilution of **TBHP-70** in physiological saline,

   Controls
   - induction treatment by intradermal injections of FCA/isotonic saline 1:1, the vehicle alone, and FCA/vehicle 1:1, followed one week later by topical application of physiological saline alone, and
   - challenge treatment, 14 days after the last induction, by topical application of a 3% test dilution of **TBHP-70** in physiological saline.

3. The challenge treatment with **TBHP-70** caused positive signs of sensitization in six of the ten test animals. On the basis of the results obtained under the conditions of this study and according to the EC-standards (mentioned in EEC Directive 93/21/EC and published in the Official Journal of the European Communities, L 110 A, Volume 36, 4 May 1993), it was concluded that **TBHP-70** is a sensitizer.

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## Statement of GLP compliance

We, the undersigned, hereby declare that this report constitutes a true and complete representation of the procedures followed and of the results obtained in this study by TNO Nutrition and Food Research, and that the study was carried out under our supervision.
The study was carried out in accordance with the OECD Principles of Good Laboratory Practice of 1997 (Organization for Economic Cooperation and Development, Paris. ENV/MC/CHEM (98)17).

M.K. Prinsen
(Study director) Date

Dr R.A. Woutersen
(Management; Head, Department of General Toxicology) Date

## Quality Assurance Statement

On : Sensitization study with **TBHP-70** in guinea pigs (maximization test)
Report Number : V3413/07
Date : July 2001

The protocol and amendment of this study was inspected as follows:

| Date of inspection: | Date of report: |
|---|---|
| 19 June 2001 | 19 June 2001 |

The experimental phase of this study was inspected by the Quality Assurance Unit as follows:

| Date of inspection: | Date of report: |
|---|---|
| 26 June 2001 | 26 June 2001 |

This report was audited as follows:

| Dates of audit: | Date of report: |
|---|---|
| July 2001 | July 2001 |

I, the undersigned, hereby declare that this report provides an accurate record of the procedures employed and the results obtained in this study; all inspections were reported to the study director and the management on the dates indicated.

Ing. S. Kanhai
(Quality Assurance Auditor)

Date:

## Testing facility

The toxicity study was conducted by:
TNO Nutrition and Food Research
Department of General Toxicology
Utrechtseweg 48, P.O. Box 360, 3700 AJ ZEIST, the Netherlands
Telephone +31 30 69 44 144; Telefax +31 30 69 60 264

## Contributors

| | |
|---|---|
| Study Director | : Mr. M.K. Prinsen |
| Dep. Study Director | : Dr M.J. Appel |
| Management | : Dr R.A. Woutersen |
| Head of Animal Care | : Mr. G. van Beek |

# 1 Introduction

At the request of Lyondell Chemical Europe, Inc., Lyondell Chemical House, Bridge Avenue, Maidenhead, Berkshire SL6 1YP, Great Britain, a sample of the title substance was examined for possible sensitization potential in guinea pigs by means of the maximization test.

# 2 Experimental

## 2.1 Test substance

| | | |
|---|---|---|
| Date of receipt | : | 8 June 2001 |
| Name | : | TBHP-70 |
| Batch no. | : | SGS REF 534326 |
| General appearance | : | clear colourless liquid |
| Quantity | : | 1 glass bottle with 250 ml |
| Chemical name | : | tertiary butyl hydroperoxide solution (70%) |
| CAS Reg. No. | : | 75-91-2 |
| TNO internal reference no. | : | 0100AF |
| Storage conditions | : | in the dark in a safety cabinet at ambient room temperature. |

## 2.2 Animals and housing conditions

| | | |
|---|---|---|
| Species | : | SPF-bred albino guinea pigs (Dunkin Hartley). |
| Supplier | : | David Hall, England |

| | | |
|---|---|---|
| Sex and age | : | males, circa 3 weeks (ordered) |
| Date of arrival | : | 20 June 2001 |
| Start date of study | : | 26 June 2001 |
| Quarantine/acclimatization period | : | 6 days |
| Termination date of study | : | 20 July 2001 |
| Identification | : | earmarking with tattoo |
| Caging | : | in a mobile battery, containing 6 cages; maximal 10 animals per cage. |
| Lighting | : | 12 hours light/12 hours dark cycle |
| Temperature during testing | : | 22 ± 3°C |
| Humidity during testing | : | 30-70% (incidentally up to 100% for a short period, because of wet cleaning of the room or meteorological circumstances). |
| Ventilation | : | ca 10 air changes/hour |
| Diet | : | standard laboratory diet *ad libitum*. Each batch of this diet is analyzed by the supplier (SDS Special Diets services, Whitham, England) for the nutrients and contaminants and the results are available upon request. Tap water (Hydron) *ad libitum*. Results of routine physical, chemical and microbiological examination of drinking water as conducted by the supplier are available upon request. |

## 2.3 Experimental design

The study was carried out at the testing facilities of the TNO Nutrition and Food Research, Utrechtseweg 48, 3704 HE Zeist, the Netherlands, according to protocol P3413/07, approved by the study director on 30 May 2001.
The procedure employed was in accordance with:

- OECD guideline no. 406, Skin Sensitization, adopted July 17, 1992, and
- EC Directive 96/54/EC, Annex IV C: B.6. Skin sensitization, dated 30 September 1996.

The study consisted of an induction treatment, followed by a resting period of 14 days, which preceded the challenge treatment.

### 2.3.1 Preliminary tests

The irritation response to intradermal injection of various concentrations of the test substance was examined in 2 guinea pigs. A sufficiently large area of the flanks was clipped free from hair with electric clippers. Amounts of 0.1 ml of the selected concentrations were applied by intradermal injection. Circa 24 hours after injection, the animals were examined for signs of irritation. A concentration causing slight to moderate irritation but otherwise well-tolerated by the animals, is usually taken for intradermal injection of the test substance in the induction phase of the main study.

The irritation response to topical treatment of various concentrations of the test substance was examined in 2 other guinea pigs. The flanks of each of the animals were clipped free from hair with electric clippers. Patches (Silverpatch, v.d. Bend B.V., Brielle, the Netherlands) were loaded with the test material and placed on the clipped skin of each animal, and covered with a piece of hypoallergenic paper bandage (Leukopor) that was secured by elastic adhesive bandage (Tensoplast), wound around the torso of the animal. The dressing was left in place for ca 24 hours. Circa twenty-four and 48 hours after removal of the dressing, the animals were examined for signs of skin irritation. A concentration causing slight to moderate skin irritation is usually chosen for topical induction and a non-irritant concentration for topical challenge.

### 2.3.2 Main study

Fifteen male guinea pigs were randomly divided into two groups, viz. one test group of 10 males and one control group of 5 males. The animals were weighed one day before the study was initiated and at the completion of the study.

#### 2.3.2.1 Induction

Induction was effected in two different ways, firstly by intradermal injections and secondly, one week later, by topical application over the injection sites.

a. Intradermal injections

For this purpose an area of about 24 $cm^2$ of dorsal skin in the scapular region was clipped free from hair with electric clippers. Pairs of intradermal injections (0.1 ml each) were made simultaneously in the clipped area as shown in Figure 1.

The following preparations were injected:

test animals

- two injections with Freund's Complete Adjuvant (FCA) and saline (1:1),
- two injections with the selected test concentration,
- two injections with the selected test concentration in FCA (1:1),

control animals

- two injections with FCA/saline (1:1),
- two injections with the diluent,
- two injections with FCA/diluent (1:1).

Skin readings were made at ca 24 hours after the treatment.

b. Topical application

Six days after the intradermal injections, the dorsal skin in the scapular region of all test and control animals was closely clipped again. On the following day, the induction by topical application was made in this region. The test animals were treated as follows:

A circa 2 x 4 cm patch of Whatman No. 3 MM filter paper was loaded with the selected concentration of the test substance. The loaded patch was placed over the sites of the intradermal injections and was secured as described in section 2.3.1. The dressing was left in place for ca 48 hours. The control animals were similarly treated with the vehicle alone. Skin readings were made directly after removal of the patches.

#### 2.3.2.2 Challenge

The topical challenge with the test substance was carried out 14 days after the topical induction as follows:
An area of circa 5 x 5 cm one flank of each test and control animal was clipped free from hair. A patch was loaded with the test concentration selected. Subsequently, the loaded patch was placed on the clipped area of the flank of each test and control animal. The patch was covered with Leukopor bandage, and held in place by Tensoplast for ca 24 hours. Skin readings were made at ca 24 and 48 hours after removal of the patch.

## 2.4 Scoring and evaluation of the results

The skin reactions were evaluated and scored according to the scale as given in Appendix 1. The controls should not show any skin reaction. If so, the severity and type of reaction was taken into account upon evaluation of effects of the skin reactions exhibited by the test animals. In absence of skin reactions on the test site challenged with the test substance of the controls, the observed skin reactions on the challenged test site of the test animals were considered as signs of sensitization. If skin reactions are observed on the test site of the controls challenged with the test substance or with the vehicle alone or if skin reactions occur on the test site of the test animals challenged with the vehicle alone, the difference in type, severity, and persistence of these reactions were evaluated in order to establish whether or not the skin reactions of the test animals were indeed signs of sensitization. The positive results were evaluated according to the EC-standards (as published in the Official Journal of the European Communities, L 110 A, Volume 36, 4 May 1993), which states that a substance is considered a sensitizer if 30% or more of the test animals show a positive reaction.

## 2.5 Retention of records, samples and specimens

All raw data and the master copy of this report are filed in the archives of the TNO Nutrition and Food Research and will be retained in the archives for a period of at least fifteen years after reporting of the study. Unless otherwise agreed, remaining test substance will be retained for at least six months after submission of the report.

## 2.6 Deviations from the protocol

The supplier of the animals was David Hall, England. No other deviations of the protocol occurred.

# 3 Results

## 3.1 Preliminary tests

The results of the preliminary irritation experiment are given in Table 1.
The degree of irritation observed after intradermal treatment with a 1% test concentration was considered suitable for intradermal treatment during the induction phase. A 30% test concentration was considered acceptable for topical treatment during the induction phase and a 3% concentration was considered suitable for the challenge phase of the study. The concentrations of 1, 3 and 30% of TBHP-70 are equivalent to 0.7, 2.1 and 21% of TBHP.
Since the 30% test concentration selected was irritating, the induction test site was not pretreated with a 10% sodium lauryl sulfate solution (SLS) in vaseline.

## 3.2 Main study

On 5 July 2001, one control showed a prolaps of the rectum because of the 48-hour application of the bandage and was subsequently sacrificed. All other animals remained in good health during the experimental period.

### 3.2.1 Induction

The individual scores of the skin reactions made during the induction phase of the study are given in Appendices 2.1 and 2.2. The intradermal injections generally caused the following skin reactions:

test animals
- FCA/saline (1:1): moderate erythema,
- selected test concentration: no skin reactions,
- selected test concentration in FCA/diluent (1:1): moderate erythema,

control animals
- FCA/saline (1:1): moderate erythema,
- vehicle: no skin reactions,
- FCA/diluent (1:1): moderate erythema.

After the topical application of the vehicle, slight erythema was observed in one control. After the 48-hour topical application of the selected test concentration, slight erythema was observed in 5 test animals.

### 3.2.2 Challenge

The results of the challenge treatment are given in Table 2.
After the challenge treatment with the 3% test concentration of TBHP-70, slight, moderate or severe erythema was observed in six test animals at 24 hours after challenge. At 48 hours after challenge, the 6 test animals showed moderate or severe erythema at the test site. The controls did not show any skin reactions.

# 4 Conclusion

The challenge treatment with **TBHP-70** caused distinct signs of hypersensitivity in 6 out of the 10 test animals. On the basis of this result it is concluded that, under the conditions of this test and according to the EC-standards (mentioned in EEC Directive 93/21/EC and published in the Official Journal of the European Communities, L 110 A, Volume 36, 4 May 1993), **TBHP-70** is a sensitizer.

# 5 Sensitivity of the test system

The sensitivity of this test system was checked by means of a positive control study with formaldehyde (37%). Formaldehyde was selected as a reference because of its known weak sensitization potential in the guinea pig maximization test. The results of this positive control study, performed in May/June 2001, are given in Annex 1 and 2 at the end of this report. The challenge treatment with a 10 and 3% test dilution of formaldehyde (37%) induced positive reactions in, respectively, 100 and 60% of the test animals, whereas no skin reactions were observed in the controls. Therefore, it was concluded that the experimental design and the strain of guinea pigs used for routine sensitization testing are suitable to detect possible sensitization potential of test materials.

# 6 Literature

- Draize, J.H., G. Woodard and H.O. Calvery J. Pharmacol. Exp. Ther. 82 (1944) 377-390.
- EC Directive 96/54/EC, Annex IV C: B.6. Skin sensitization, dated 30 September 1996
- EC standards, EEC-Directive 93/21/EC, Official Journal of the European Communities, L 110 A, Volume 36, 4 May 1993.
- Magnusson, B. and A.M. Kligman J. Invest. Dermat. 52 (1969) 168-276.
- Magnusson, B. and A.M. Kligman Allergic contact dermatitis in the guinea pig; identification of contact allergens, Thomas, Springfield, Illinois, 1970.
- OECD Guidelines for Testing of Chemicals, nr. 406, adopted 17 July 1992.

## Table 1

Preliminary irritation experiment: Scores obtained after topical applications of various concentrations of **TBHP-70** in physiological saline

Intradermal treatment: Scores at ca 24 h after injection

| Animal no. | Concentration | | | |
|---|---|---|---|---|
| | 3% | 1% | 0.3% | 0.1% |
| 168 | 1n | 1 | 0 | 0 |
| 170 | 1n | 1 | 0 | 0 |

The grading system is given in Appendix 1.

Topical treatment: Scores at ca 24 and 48 h after a ca 24-hour topical treatment

| Animal no. | Concentration: 30% | | 10% | | 3% | | 1% | |
|---|---|---|---|---|---|---|---|---|
| | 24 h | 48 h | 24 h | 48 h | 24 h | 48 h | 24 h | 48 h |
| 176 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 178 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |

The grading system is given in Appendix 1.

Selection of concentrations:
- Intradermal induction : 1% in physiological saline
- Topical induction : 30% in physiological saline
- Topical challenge : 3% in physiological saline

## Table 2

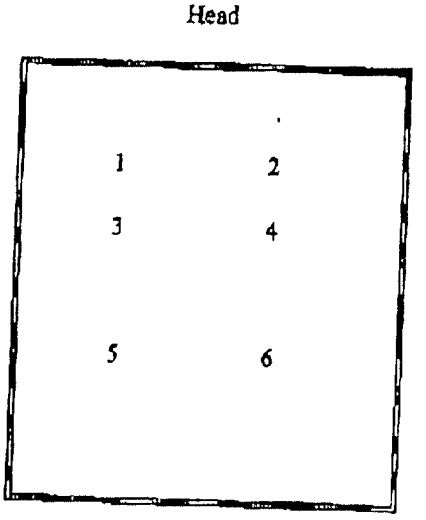
Dermal reactions elicited by the challenge application of a 3% dilution of TBHP-70 in physiological saline

| Skin scores obtained at 24 and 48 hours after removal of the dressing | | |
|---|---|---|
| Animal no. | 24 hours | 48 hours |
| **Control group** | | |
| 22 | 0 | 0 |
| 24 | 0 | 0 |
| 26 | 0 | 0 |
| 30 | 0 | 0 |
| **Test group** | | |
| 2 | 1 | 2 |
| 4 | 2 | 3 |
| 6 | 2 | 3 |
| 8 | 0 | 0 |
| 10 | 3 | 3 |
| 12 | 0 | 0 |
| 14 | 2 | 2 |
| 16 | 0 | 0 |
| 18 | 2 | 3 |
| 20 | 0 | 0 |

The grading system is given in Appendix 1.

## Figure 1

Position of intradermal injections in the shoulder region of the guinea pig made in the induction phase of the study




1 + 2 = 0.1 ml Freund's Complete Adjuvant (FCA) and saline (1:1), control and test animals

3 + 4 = 0.1 ml of the selected concentration of the test substance in a suitable carrier (test animals), or the carrier alone (controls); no injections are made if the undiluted test substance is given to the test animals

5 + 6 = 0.1 ml of the selected dilution of the test substance in FCA 1:1 (test animals), or FCA and the carrier 1:1 (controls: if the undiluted test substance or a dilution in saline is used, just FCA/saline 1:1 will be given)

# Appendix 1

READING OF SKIN REACTIONS

**1. Reading of skin reactions after intradermal injection**

0 = no visible changes
1 = slight erythema
2 = moderate erythema
3 = severe erythema; eschar formation (injuries in depth)

a = abscesses; n = necrosis; i = incrustation

**2. Reading of skin reactions after topical application (Magnusson)**

0 = no visible change
1 = discrete or patchy (slight) erythema
2 = moderate and confluent erythema
3 = intense (severe) erythema and swelling

## Appendix 2.1

Individual body weights and skin reactions observed in controls during the induction phase of the study with **TBHP-70**

| Animal no. | Body weight (g) | | Intradermal scores at injection site no.: | | | | | | Topical |
|---|---|---|---|---|---|---|---|---|---|
| | start | end | 1 | 2 | 3 | 4 | 5 | 6 | |
| 22 | 231 | 407 | 2 | 2 | 1 | 1 | 2 | 2 | 0 |
| 24 | 221 | 369 | 2 | 2 | 1 | 1 | 2 | 2 | 1 |
| 26 | 215 | 374 | 2 | 2 | 0 | 0 | 2 | 2 | 0 |
| 28 | 223 | -[1] | 2 | 2 | 0 | 0 | 2 | 2 | - |
| 30 | 238 | 456 | 2 | 2 | 1 | 1 | 2 | 2 | 0 |

[1] = animal killed because of a prolaps of the rectum

The grading system is given in Appendix 1.

injection no. 1 and 2 : FCA/saline (1:1)
injection no. 3 and 4 : vehicle
injection no. 5 and 6 : FCA/vehicle (1:1)

topical application : physiological saline

## Appendix 2.2

Individual body weights and skin reactions observed in test animals during the induction phase of the study with TBHP-70

| Animal no. | Body weight (g) | | Intradermal scores at injection site no.: | | | | | | Topical |
|---|---|---|---|---|---|---|---|---|---|
| | start | end | 1 | 2 | 3 | 4 | 5 | 6 | |
| 2 | 235 | 418 | 2 | 2 | 0 | 0 | 2 | 2 | 1 |
| 4 | 262 | 404 | 2 | 2 | 0 | 0 | 2 | 2 | 0 |
| 6 | 234 | 325 | 2 | 2 | 0 | 0 | 2 | 2 | 1 |
| 8 | 223 | 379 | 2 | 2 | 0 | 0 | 2 | 2 | 0 |
| 10 | 239 | 361 | 2 | 2 | 0 | 0 | 2 | 2 | 0 |
| 12 | 231 | 359 | 2 | 2 | 0 | 0 | 2 | 2 | 0 |
| 14 | 219 | 372 | 2 | 2 | 0 | 0 | 2 | 2a | 1 |
| 16 | 262 | 430 | 2 | 2 | 0 | 0 | 2 | 2 | 0 |
| 18 | 229 | 362 | 2 | 2 | 0 | 0 | 2 | 2 | 1 |
| 20 | 229 | 409 | 2 | 2 | 0 | 0 | 2 | 2 | 1 |

The grading system is given in Appendix 1.

injection no. 1 and 2: FCA/saline (1:1)
injection no. 3 and 4: selected test concentration
injection no. 5 and 6: selected test concentration in FCA (1:1)

topical application : selected test concentration

## Annex 1.1

Individual skin reactions observed in controls during the induction phase of a positive control study with Formaldehyde (study performed in May/June 2001, according to protocol 42113/01.01)

| Animal no. | Body weight (g) | | Intradermal scores at injection site no.: | | | | | | Topical |
|---|---|---|---|---|---|---|---|---|---|
| | start | end | 1 | 2 | 3 | 4 | 5 | 6 | |
| 162 | 296 | 455 | 2 | 2 | 0 | 0 | 2 | 2 | 1 |
| 164 | 269 | 423 | 2 | 2 | 0 | 0 | 2 | 2 | 1 |
| 166 | 251 | 393 | 2 | 2 | 0 | 0 | 2 | 2 | 1 |

The grading system is given in Appendix 1.

injection no. 1 and 2 : FCA/saline (1:1)
injection no. 3 and 4 : saline
injection no. 5 and 6 : FCA/saline (1:1)

topical application : patches moistened with saline (test sites pretreated with 10% SLS)

## Annex 1.2

Individual skin reactions observed in test animals during the induction phase of a positive control with Formaldehyde (study performed in May/June 2001, according to protocol 42113/01.01)

| Animal no. | Body weight (g) | | Intradermal scores at injection site no.: | | | | | | Topical |
|---|---|---|---|---|---|---|---|---|---|
| | start | end | 1 | 2 | 3 | 4 | 5 | 6 | |
| 152 | 292 | 427 | 2 | 2 | 1 | 1 | 2a | 2a | 2 |
| 154 | 242 | 372 | 2 | 2 | 1 | 1 | 2a | 2a | 1 |
| 156 | 272 | 444 | 2 | 2 | 1 | 1 | 2a | 2a | 1 |
| 158 | 276 | 417 | 2 | 2 | 1 | 1 | 2a | 2a | 2 |
| 160 | 255 | 410 | 2 | 2 | 1 | 1 | 2a | 2a | 1 |

a = abscesses

The grading system is given in Appendix 1.

injection no. 1 and 2: FCA/saline (1:1)
injection no. 3 and 4: 0.3% test dilution of formaldehyde (37%) in saline
injection no. 5 and 6: 0.3% test dilution in FCA/saline (1:1)

topical application : 20% test dilution of formaldehyde (37%) in saline (test sites pretreated with 10% SLS)

## Annex 2.1

Dermal reactions elicited by the challenge application of a 10% dilution of formaldehyde 37% (study performed in May/June 2001, according to protocol 42113/01.01)

| Skin scores obtained at 24 and 48 hours after removal of the dressing | | |
|---|---|---|
| Animal no. | 24 hours | 48 hours |
| **Control group** | | |
| 162 | 0 | 0 |
| 164 | 0 | 0 |
| 166 | 0 | 0 |
| **Test group** | | |
| 152 | 1 | 1 |
| 154 | 0 | 1 |
| 156 | 1 | 2 |
| 158 | 2 | 3 |
| 160 | 2 | 2 |

The grading system is given in Appendix 1.

## Annex 2.2

Dermal reactions elicited by the challenge application of a 3% dilution of formaldehyde 37% (study performed in May/June 2001, according to protocol 421 13/01.01)

| Skin scores obtained at 24 and 48 hours after removal of the dressing | | |
|---|---|---|
| Animal no. | 24 hours | 48 hours |
| **Control group** | | |
| 162 | 0 | 0 |
| 164 | 0 | 0 |
| 166 | 0 | 0 |
| **Test group** | | |
| 152 | 0 | 0 |
| 154 | 0 | 0 |
| 156 | 1 | 1 |
| 158 | 2 | 2 |
| 160 | 1 | 2 |

The grading system is given in Appendix 1.